UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

IN RE COLUMBIA UNIVERSITY PATENT LITIGATION	MDL No. 1592 (MLW) This Document Relates To All Actions

EXPERT REPORT OF PROFESSOR FRANCIS H. RUDDLE SUBMITTED ON BEHALF OF THE TRUSTEESS OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

I. QUALIFICATIONS

Since 1961, I have served as a faculty member in the Department of Zoology, subsequently named the Department of Biology, and recently named the Department of Molecular, Cellular, and Developmental Biology, at Yale University in New Haven, Connecticut. I also hold a secondary faculty appointment in the Department of Genetics, Yale School of Medicine. I was promoted to full Professor in 1972, named to the Ross Granville Harrison professorship in 1983, and to the Sterling professorship in 1988. This year, I became a Senior Research Biologist, and Sterling Professor of Biology and Genetics, Emeritus. Among my research interests are somatic cell genetics, genomics, and pattern formation. My laboratory was among the first to map human genes using cell hybrids, to demonstrate the feasibility of genetically transfecting complex organisms (transgenic mice), to demonstrate the role of Hox genes in mammalian development, and to explore the role of Hox genes in animal evolution. I am an author on more than 600 publications in my field, approximately 135 of which were published in the past ten years. My curriculum vitae and a list of my publications are attached as Exhibit A.

I am very familiar with the technology at issue in this case and the state of the art at the time of the inventions claimed in U.S. Patent Nos. 4,399,216 (the "'216 patent"), 5,634,665 (the "'665 patent"), 5,179,017 (the "'017 patent") (collectively, the "original Axel Patents"), as well as U.S. Patent No. 6,455,275 (the "'275 patent"). In the late 1970s and early 1980s, my laboratory was exploring the insertion of genetic material into eukaryotic cells, and the assessment of this genetic material. The laboratories in which Richard Axel, Saul Silverstein and Michael Wigler worked and my own laboratory were therefore peers and, in a sense, competitors.

I served in the United States Air Force from 1946 to 1949. I received a Ph.D. in Cell Biology, under the direction of Dr. Morgan Harris, from the University of California, Berkeley in 1960. As an NIH postdoctoral-fellow, I studied with Dr. John Paul, Department of Biochemistry, University of Glasgow, Scotland, 1960-61. I served for ten years as chairman of the Department of Biology, Yale University. I was elected to the National Academy of Sciences in 1976. I have served as the President of the Society for Developmental Biology, the American Society of Human Genetics, and the American Society for Cell Biology. I am also a Member of the American Academy of Arts and Sciences and the Institute of Medicine of the National Academy of Science. I am a Fellow of the American Association for the Advancement of Science and the American Academy of Microbiology. I have received Honorary Degrees from Lawrence University, The Weizmann Institute, and Wayne State University. I am a founding editor of the journals *Genomics* and *Molecular Phylogenetics and Evolution*, and served as editor of numerous biological science publications, including *American Journal of Medical Genetics*, *Journal of Cell Biology*, *Molecular Biology and Medicine* and *Somatic Cell Genetics*. I am also currently Editor-in-Chief of the *Journal of Experimental Zoology*. I have served as a member of numerous governmental advisory panels.

II. OPINIONS

A. INTRODUCTION

I have been asked to determine whether certain claims of the '275 patent would have been obvious to a person of ordinary skill in the art as of February 1980 in light of the claims of the original Axel patents. My understanding is that this analysis requires me to compare each claim of the '275 patent to each claim of the earlier Axel patents, and

determine if there are any differences between such claims. If there are any differences, I am advised that I must then determine whether such differences in the later claim would have rendered it an obvious variation on the earlier claim, when considered from the perspective of a person of ordinary skill in the art working at the time the invention was made.

As part of my analysis, I have taken the following steps. I have been asked to assume for the purposes of my report that the date of the inventions claimed in the '275 patent is February 25, 1980. I have therefore considered the state of knowledge of recombinant DNA technology and related fields (e.g., molecular biology, biochemistry, genetics) as of that date. I have further reviewed the patent and prosecution records for the '216, '017, '665, and '275 patents and have studied them in order to understand and to make judgments concerning the claims in those patents. I have performed this analysis based on the knowledge that a person of ordinary skill in the art of the Axel patents would have had as of February 1980. In my opinion, a person of ordinary skill in the art would be defined as possessing a Ph.D. degree in molecular biology or related disciplines and with knowledge in cell biology, developmental biology, biochemistry and genetics at the level of a post-doctoral fellow or an assistant professor. A person of ordinary skill in the art would also have had multiple years of laboratory experience in recombinant DNA technology.

I have been asked to consider specifically whether claims 3, 5-14, and 16-19 of the '275 patent would have been obvious to a person of ordinary skill in the art as of 1980 in light of the claims of the Original Axel Patents. There are at least two novel aspects of these claims that I intend to discuss: (1) All of the claims disclose the stable incorporation of DNA II into chromosomal DNA of a host cell. (2) Claim 19 addresses the production of a "glycoprotein of interest." None of the claims of the '216, '665 or '017 patents recites these

aspects of the '275 patent. In addition, these aspects of the '275 patent would not have been obvious in light of the '216, '665 and '017 patents.[1] It is my opinion that a person of skill in the art (whether ordinary or extraordinary) would have considered claims 3, 5-14, and 16-19 of the '275 patent to be non-obvious in light of any claim of the original Axel patents and the understanding in the art as of February 1980.[2]

The general field of the Axel patents is the insertion of DNA into eukaryotic cells. This is sometimes referred to as DNA-mediated gene transfer or, more generally, as recombinant DNA technology.

The level of ordinary skill in the art covered by the Axel patents was quite low as of February 1980. Relatively few research groups were studying DNA-mediated gene transfer into eukaryotic cells and those groups working in the field were hampered by the lack of good diagnostic tools to determine the disposition of DNA once introduced. Further complicating the picture, and as discussed in greater detail below, the complex molecular processes implicated by the '275 patent, such as amplification and post-translational modification, were poorly understood in February 1980. These factors combined to create a situation in which there simply was not the critical mass of research and experience that would make it reasonable, let alone easy, to predict outcomes of experiments.

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[1] I understand that a claim of the '275 patent is not obvious over a claim of the '216, '665, or '017 patents unless the latter claim would give a person of ordinary skill in the art at the time of the invention a reasonable expectation that he or she will be successful in making the invention covered by the claim of the '275 patent.

[2] Although I have considered all the claims of the '275 for the purposes of forming an opinion about the meaning of those claims, I have been asked to analyze for obviousness all claims in the '275 patent except for claims 1, 2, 4, 15 and 20.

B. PROKARYOTIC VERSUS EUKARYOTIC GENE EXPRESSION SYSTEMS

The two major classes of cells are prokaryotes and eukaryotes. Prokaryotes are simple organisms such as bacteria that have relatively small genomes and lack a distinct nucleus. Eukaryotes are more complex with larger genomes and possess an organized nucleus that separates the DNA from the rest of the cell. In eukaryotic cells, most DNA resides on chromosomes. The chromosomes are the genetic blueprint for the cell.

In both eukaryotic and prokaryotic cells, DNA serves as a repository of genetic information, and helps determine the precise structure and function of molecules produced by the cell. DNA "encodes" polypeptides (polymers of amino acids) by the processes of transcription and translation. In transcription, the cell reads the sequence of a DNA strand, called a gene, that contains the coding for a particular polypeptide. This process results in the creation of a strand of messenger RNA (or mRNA), sometimes referred to as a "transcript." The linear sequence information in mRNA specifies the order of amino acids in a polypeptide. In translation, the cell reads the mRNA transcript and creates a specific sequence of amino acids (a polypeptide) based on the sequence of the transcript. After transcription and translation, the cell frequently performs certain post-translational processing on the polypeptide. Post-translational processing transforms the polypeptide into its ultimate three-dimensional structural and functional state.

The processing machinery for transcription, translation, and post-translational processing in prokaryotes and eukaryotes is related evolutionarily, but is highly differentiated as a result of a more than one billion years of divergence between prokaryotes and eukaryotes from a primitive common ancestor. Some of the differences between prokaryotic and eukaryotic cells include the following: (i) The promotor and other

regulatory DNA sequences, which are used to instruct a cell where and when to start transcribing DNA to make mRNA, are different in eukaryotic and prokaryotic cells. Prokaryotic cells generally do not recognize the regulatory sequences of eukaryotic DNA (and in particular, mammalian DNA). (ii) Eukaryotic genes generally possess non-coding DNA sequences (introns) interspersed among DNA coding sequences (exons). The intron sequences are not intended to be translated into amino acids. Only the exon sequences are intended to be translated. The appropriate removal of introns and the joining (splicing) of exons gives rise to authentic mRNA transcripts that encode the intended polypeptide. This splicing process requires dedicated machinery present in eukaryotes, but generally absent in prokaryotes.

Because prokaryotic cells generally lack the proper processing machinery to transcribe and translate a eukaryotic gene correctly, a scientist working in 1980 would generally have had to create a modified version of that gene in order for the prokaryotic cell to process it correctly. One significant benefit of the use of eukaryotic cells is that it enables a researcher to obtain transcription and translation of a foreign eukaryotic gene without the need to modify the gene.

Up to this point, I have focused on transcription and translation of DNA to produce a polypeptide. However, there are additional functions that a cell performs. Many newly-translated polypeptides in eukaryotic cells undergo one or more modifications – referred to as post-translational modifications – before they are in their final form. One example of a post-translational modification involves the cleavage of certain unnecessary amino acid sequences from the polypeptide. Another example is the creation of special types of bonds

between parts of the polypeptide. Still another example is the attachment of sugar molecules to the polypeptide. This last process is known as glycosylation.

The process of post-translational modification was not well-understood in February 1980 and to this day significant questions about it persist. Generally, as an mRNA is translated into a polypeptide, the host cell recognizes signaling structures on the polypeptide which instruct the cell to move the polypeptide into the processing apparatus in the cell. The two primary parts of the processing apparatus are the endoplasmic reticulum (ER) and the golgi apparatus (golgi). As the polypeptide moves through the processing apparatus, post-translational modifications occur.

One of the complex processing steps that can occur in certain polypeptides involves the attachment of sugar molecules (i.e., gylcosylation). Many different enzymes and structures must act in concert to move a polypeptide through the processing apparatus in a host cell and to add sugar molecules. It is important to note that the sugar molecules on a polypeptide can be built up over time and each step in the process is controlled by a specific enzyme. Therefore, if a specific enzyme is missing, one part of the sugar molecules may not be added to the polypeptide. In addition, if a cell stops a polypeptide from progressing through the processing apparatus as the result of certain signaling structures, glycosylation and other post-translational modifications that would normally occur may fail to take place.

Prokaryotic cells are greatly restricted in their ability to perform the post-translational modifications that occur in eukaryotic cells. The inability to perform the necessary post-translational modifications of eukaryotic cells is a significant deficiency from the standpoint of recombinant DNA technology. In February 1980, researchers understood that there was a correlation between structure and function in eukaryotic polypeptides.

Because the structure of a molecule influences function, researchers understood that if they were going to produce eukaryotic polypeptides in a host cell, it would be desirable to produce them with the same structure (or as close to the same structure as possible) that they would ordinarily possess in nature. An example of this is human insulin. When the insulin gene is initially expressed in a human cell, it is made as a precursor containing certain amino acid sequences that render it inactive. It is only once these amino acid sequences are removed during post-translational modification that insulin becomes active. Researchers understood that it was desirable to obtain the active form of insulin, that is to say, the insulin with the precursor amino acids removed.

C. GLYCOSYLATION IN EUKARYOTIC EXPRESSION SYSTEMS

In February 1980, a person of ordinary skill in the art (or for that matter a person of extraordinary skill), with knowledge of the original Axel claims, would not have considered it obvious to construct a eukaryotic cell (and in particular a mammalian or Chinese Hamster Ovary cell) with the characteristics of the host cell of claim 19 of the '275 patent that was competent to produce the "glycoprotein of interest" of claim 19, and culture it under appropriate conditions to obtain that glycoprotein. I base these conclusions on at least the following facts and circumstances:

Signal Structure Recognition: In February 1980 there were data suggesting that a eukaryotic cell recognizes signaling structures located on a polypeptide and that these signals instruct the cell regarding the transport of the polypeptide through the different parts of the cell's processing apparatus. Data also suggested that there were signaling structures that could stop or redirect the transport of a polypeptide in a host cell, and thereby stop or redirect its post-translational processing. Unfortunately, there was not a lot known about how

differences in the signaling structures affected what a cell thought it was supposed to do.[3] A worker considering the prospects for post-translational modification of a foreign polypeptide (i.e., the polypeptide product of a foreign DNA) would have been very concerned about whether the host cell possessed the appropriate mechanism to process the foreign polypeptide's signaling structures properly.

Specific Glycosylation Structures: An understanding of glycoprotein synthesis was at best rudimentary in February 1980.[4] Glycoproteins are made up of amino acids and carbohydrates (e.g., sugars). The process of creating the sugar polymers that are added to the amino acids in glycosylation is extraordinarily complex. A variety of different sugars may be combined into a chain that is attached to a polypeptide. In addition, multiple chains of sugar molecules may be attached to the same polypeptide. Each sugar may exist in a number of different forms, and these in turn may be joined to each other by a variety of different linkages. Thus, even a short chain of a few sugar molecules may be generated in many different configurations. Finally, the sugar polymers must be bound properly to the appropriate amino acid on the polypeptide. Each of these steps generally requires the presence of a specific enzyme expressed within a particular cellular compartment. The

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[3] Devillers-Thiery, A. *et al.*, "Homology in amino-terminal sequence of precursors to pancreatic secretory proteins," *Proc. Nat. Acad. Sci. U.S.A.* 72(12):5016-5020 (1975); Lingappa, R., *et al.*, "A signal Sequence for the Insertion of a Transmembrane Glycoprotein," *J. Biol. Chem.* 253(24): 8667-8670 (1978); Davis, B.D. *et al.*, "The mechanism of protein secretion across membranes," *Nature* 283: 433-438 (1980).

[4] In February 1980, the field of glycobiology was poorly developed and dimly perceived. As pointed out in the Foreword to *Essentials of Glycobiology* by Professor Stuart Kornfeld, a leading researcher in the field, "Advances in this area [glycobiology] continued at a steady rate during most of this century, but the past 20 years has witnessed an unparalleled explosion of new knowledge that has transformed the field." Kornfeld, S., "Foreword," in *Essentials of Glycobiology*, at vii (Varki, A. *et al.*, eds. 1999).

regulation of this system of biosynthesis was poorly understood in February 1980, and continues to be a subject of research today.

Most of the steps in glycosylation, and in post-translational modification generally, are controlled by highly specialized enzymes in the host cell.[5] These enzymes are produced by genes in the host cell.[6] A cell that has been transformed with foreign DNA encoding a polypeptide does not necessarily possess the specific enzymes required to glycosylate the foreign polypeptide during post-translational modifications.

For example, in February 1980, scientists could have had no confidence that a CHO cell had the same specific types of enzymes and other structures required to perform post-translational processing on a foreign polypeptide. To the contrary, there was substantial experimental evidence that even slight genetic differences in a cell could have a profound impact on the type of glycosylation that is performed. Before February 1980, researchers in the field published a number of papers on a group of CHO mutant cells. These cells lacked certain very specific processes that normally exist in CHO cells. Because of the lack of these very specific processes, the glycosylation performed by these mutant CHO cells was

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[5] Beyer, T. A. *et al.*, "Biosynthesis of Mammalian Glycoproteins," *J. Biol. Chem.*, 254 (24):12531-12541 (1979); Ginsburg, V. *et al.*, "Structure and Function of Surface Components of Mammalian Cells," in *Structure and Function of Biological Membranes* 439-459 (Rothfield ed. 1971); Spiro, R.G., "Glycoproteins," in *Adv. Protein Chem.* 27:349-467 (1973); Marshall, R.D., "Glycoproteins," in *Annual Review of Biochemistry* Vol. 41, 673-702 (Snell *et al.*, eds. 1972); Gottschalk, A., "Biosynthesis of glycoproteins and its relationship to heterogeneity," *Nature*, 222:452-454 (1969).

[6] Ginsburg, V. *et al.*, "Structure and Function of Surface Components of Mammalian Cells," in *Structure and Function of Biological Membranes*, at 449-50 (Rothfield, ed. 1971) ("From a genetic viewpoint, the carbohydrate structures of cell surfaces can be considered to be secondary gene products in that the primary gene products are enzymes and these enzymes, working in concert, determine what specific structures are formed.").

different from that performed by non-mutant CHO cells.[7] Simply put, in February 1980 scientists understood that small differences in the enzymes available in a cell could have significant effects on the ability of a cell to perform post-translational modifications.

Effects of Culturing on Cells: The cells that scientists normally use in recombinant DNA technology are specially adapted for research. These cells can divide for many generations without stopping. Cells adapted to culturing for long periods in a laboratory can be very different from the body cells from which they were originally derived. Once a cell is removed from its natural environment and takes on the ability to divide for long periods of time, the functions that cell performs may change, for example because of changes in the number and/or organization of the cell's chromosomes. This being the case, one could not have reasonably predicted in February 1980 whether the ability of a cell to perform a particular function would be retained, modified, or lost in the course of continuous propagation in culture.[8]

Species and Cell Type Differences In Glycosylation: As a general rule, not every cell in an organism is the same. For example, complex mammalian organisms are made up of a plethora of specialized cells that, although genetically similar, are very different in terms of

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[7] *See* Gottlieb, C. *et al.*, "Deficient Uridine Diphosphate-*N*-acetylglucosamine: Glycoprotein *N*-Acetylglucosaminyltransferase Activity in a Clone of Chinese Hamster Ovary Cells with Altered Surface Glycoproteins," *J. Biol. Chem.*, 250(9):3303-3309 (1975); Gottlieb, C. *et al.*, "Isolation of a clone of Chinese hamster ovary cells deficient in plant lectin-binding sites," *Proc. Nat. Acad. Sci. U.S.A.* 71(4):1078-1082 (1974); Stanley, P. *et al.*, "Selection and Characterization of Eight Phenotypically Distinct Lines of Lectin-Resistant Chinese Hamster Ovary Cells," *Cell* 6:121-128 (1975).

[8] Sirica, A.E. *et al.*, "Fetal phenotypic expression by adult rat hepatocytes on collagen gel/nylon meshes," *Proc. Natl. Acad. Sci. USA* 76(1): 283-287 (1979); Hausman, S.J. *et al.*, "Alteration of immunoglobulin phenotype in cell culture-adapted lines of two mouse plasmacytomas," *J. Exp. Med.* 142(4):998-1010 (1975); Littlefield L.G. *et al.*,

the roles they play in an organism.[9] Different cell types in an organism frequently express different types of polypeptides, and because of this, the types of post-translational modifications they normally perform are different. A person of ordinary skill in the art in February 1980 would have been extremely concerned about whether a host cell that is not known to ordinarily perform a particular post-translational modification on a particular polypeptide (i.e., the foreign polypeptide) would have the capability to perform the necessary post-translational modifications to that foreign polypeptide. In fact, there would have been substantial concern that a host cell might not recognize what to do with a foreign polypeptide, unless there were experimental data that established otherwise.[10]

Moreover, there was experimental evidence by February 1980 suggesting that different cells produce different glycosylation patterns on the same polypeptide.[11] In fact,

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"Observations of de novo clones of cytogentically aberrant cells in primary fibroblast cell strains from phenotypically normal women," *Am. J. Hum. Genet.* 27(2):190-197 (1975).

[9] *See, e.g.*, Muramatsu, T. *et al.*, "Carbohydrate structures and cell differentiation: Unique properties of fucosyl-glycopeptides isolated from embryonal carcinoma cells," *Proc. Natl. Acad. Sci. U.S.A.* 75(5):2315-2319 (1978); Muramatsu, T. *et al.*, "Charaterization of glycopeptides isolated from membranes of F9 emryonal carcinoma cells," *Biochim. Biophys. Acta*, 587:392-406 (1979).

[10] A related concern would be whether a researcher, in the laboratory, could supply the specific nutrients and materials a cell might require to perform a particular post-translational modification. When part of an organism, the cell might obtain the specific components from other cells in the organism or its natural environment. In a laboratory, it might not have this ability.

[11] *See, generally,* Kornfeld, R. *et al.*, "Comparative Aspects of Glycoprotein Structure," in *Annual Review of Biochemistry* Vol. 45, 217-237 (Snell *et al* eds. 1976); Moczar, E., "Differentiation of the polysaccharide side-chains of glycoproteins by a fingerprinting technique: heterogeneity of human gastric mucin," *J. Chromatogr. Biomed. Appl.*, 181:108-114 (1980); Marshall, R., "Some Observations on Why Many Proteins are Glycosylated," *Biochem. Soc. Trans.* 7(4):800-805 (1979). *See also* Clamp, J.R. *et al.*, "Heterogeneity of glycopeptides from a homogeneous immunoglobulin," *Biochem. J.*, 100:35c-36c (1966); Gottschalk, A., "Biosynthesis of glycoproteins and its relationship to heterogeneity," *Nature* 222:452-454 (1969); Spiro, R.G., "Glycoproteins," *Adv. Protein Chem.* 27:349-467 (1973).

there was evidence that an organism would sometimes produce both non-glycosylated and glycosylated forms of the same molecule.[12] Researchers at the time would have accepted that different cells may treat the same polypeptide differently for post-translational modification purposes. Because of this variability, researchers in February 1980 would not have considered there to be a reasonable likelihood of success that a given host cell would be able to reproduce a glycosylation pattern for a foreign glycoprotein.[13]

Changes In Glycosylation Patterns Impact Function: It is important to emphasize that the presence or absence of a glycosylation pattern is not just a point of academic interest. Rather, researchers in February 1980 understood that it could have significant real-world consequences. In February 1980, researchers understood that glycosylation could play essential roles in the function of a molecule.[14] For example, glycosylation appeared to play

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[12] *See, e.g.*, Marshall, R., "Some Observations on Why Many Proteins are Glycosylated," *Biochem. Soc. Trans.* 7(4):800-805 (1979).

[13] Although my report focuses on glycosylation, as noted previously there are other types of post-translation modifications. For the same reasons discussed regarding glycosylation, there would have been doubt in 1980 about whether a transformed mammalian cell could perform a particular post-translational modification other than glycosylation on a foreign polypeptide. Well past 1980, investigators treated evidence that a cell could perform a post-translational modification on a foreign polypeptide as significant. For example, Randall Kaufman published a paper in 1986, announcing that he had successfully expressed Factor IX in a CHO cell. Factor IX requires a particular post-translational modification for activity, called γ-carboxylation. Kaufman notes that: "Although CHO cells were not known previously to have the enzymatic machinery for γ-carboxylation, we have demonstrated that these cells, in the presence of vitamin K, are capable of processing the Factor IX precursor to γ-carboxylate certain glutamic acid residues to generate a biologically active Factor IX molecule." Kaufman, R.J. *et al.*, "Expression, Purification, and Characterization of Recombinant γ-Carboxylated Factor IX Synthesized in Chinese Hamster Ovary Cells," *J. Biol. Chem.* 261(21):9627 (1986).

[14] Spiro, R.G., "Glycoproteins," in *Advances in Protein Chemistry* Vol. 27, 349-467 (Anfinsen, C.B. *et al.*, ed. 1973); Olden, K. *et al.*, "Role of Carbohydrates in Protein Secretion and Turnover: Effects of Tunicamycin on the Major Cell Surface Glycoprotein of Chick Embryo Fibroblasts," *Cell*, 13:461-473 (1978); Chu, F., *et al.*, "The Effect of Carbohydrate Depletion on the Properties of Yeast External Invertase," *J. Biol Chem.* 253(24):8691-8693 (1978); Eagon, P.K. *et al.*, "Glycoprotein Biosynthesis in Myeloma

an important role in stopping an organism from destroying and/or excreting a molecule. For a molecule to perform a function in an organism, it must be able to stay in that organism intact. Glycosylation also plays a vital role in protein-protein recognition. For many biological processes to occur, two molecules must be able to recognize each other. Without proper glycosylation, a molecule's counterpart in a reaction may not be able to recognize it. What is significant about the research from the period was that it made clear that small changes in the pattern of post-translational modification could have a significant impact on the function of a molecule.[15]

Recognizing that cells of different species and different cells from the same species glycosylate differently, and recognizing that small differences in glycosylation profoundly influence function, researchers in February 1980 would have seriously questioned whether a given host cell (e.g., a CHO cell) could properly glycosylate a given foreign polypeptide. In fact, these doubts persisted well beyond 1980. Charles Weissmann, one of the leading figures in the production of recombinant molecules, expressed concern in 1983 that differences in glycosylation patterns between a human cell and a CHO cell could

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Cells," *J. Biol. Chem.* 252(7):2372-2383 (1977); Loh, Y. *et al.*, "The Role of the Carbohydrate in the Stabilization, Processing, and Packaging of the Glycosylated Adrenocorticotropin-Endorphin Common Precursor in Toad Pituitaries," *Endocrinology* 105(2):474-486 (1979).

[15] Winkelhake, J.L. *et al.*, "Aglycosylantibody," *J. Biol. Chem.*, 251(4):1074-1080 (1976); Sodetz, J. *et al.*, "Carbohydrate on Human Factor VIII/von Willebrand Factor," *J. Biol. Chem.* 253(20):7202-7206 (1978); Baynes, J. W. *et al.*, "Effect of Glycosylation on the *in Vivo* Circulating Half-life of Ribonuclease," *J. Biol. Chem.*, 251 (19): 6016-6024 (1976); Briggs, D.W. *et al.*, "Hepatic clearance of intact and desialylated erythropoietin," *Am. J. Physiol.*, 227(6):1385-1388 (1974); Morell, A.G. *et al.*, "The role of sialic acid in determining the survival of glycoproteins in the circulation," *J. Biol. Chem.*, 246(5):1461-1467 (1971); Schlesinger, P.H. *et al.*, "Plasma clearance of glycoproteins with terminal mannose and *N*-Acetylglucosamine by liver non-parenchymal cells," *Biochem J.*, 176:103-109 (1978).

compromise the functionality of recombinant molecules produced in CHO cells.[16] Indeed, it was only after February 1980 that scientific journals began publishing papers establishing that a host cell has properly glycosylated a foreign polypeptide.[17] For example, in 1988, a scientific publication dealing with the glycosylation of a foreign polypeptide states that "This paper proved, for the first time, that recombinant technique can produce glycoprotein hormone whose carbohydrate structures are common to the major sugar chains of the native one."[18]

Analysis Of The Claims

Claim 19 of the '275 patent recites a host cell that is competent to produce a "glycoprotein of interest" and has been cultured under appropriate conditions such that it comprises the molecule. Below is a comparison of claim 19 and those claims of the '216, '665 and '017 patents cited by plaintiffs in their double-patenting charts against claim 19. However, it is important to emphasize that I believe that claim 19 is not obvious in light of any claim of the '216, '665 and '017 patents, not just those cited by the plaintiffs.

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[16] Haynes, J. *et al.*, "Constitutive, Long-term Production of Human Interferons by Hamster Cells Containing Multiple Copies of a Cloned Interferon Gene," *Nucleic Acids Res.*, 11(3):703 (1983) ("As it is not known whether glycosylation in hamster and human cells leads to identical structures it may be more appropriate to generate and use a human . . . cell line for the production of human glycoproteins."). *See also* Kaufman, R.J., "High Level Production of Proteins in Mammalian Cells," in *Genetic Engineering: Principles and Methods*, 9:155-198 (1987).

[17] McCormick, F. *et al.*, "Inducible Expression of Amplified Human Beta Interferon Genes in CHO Cells," *Mol. Cell Biol.*, 4(1):166-172 (1984); Kaufman, R.J. *et al.*, "Coamplification and Coexpression of Human Tissue-Type Plasminogen Activator and Murine Dihydrofolate Reductase Sequences in Chinese Hamster Ovary Cells," *Mol. Cell. Biol.*, 5(7):1750-1759 (1985).

[18] Takeuchi, Makoto, *et al.* "Comparative Study of the Asparagine-linked Sugar Chains of Human Erythropoietins Purified from Urine and the Culture Medium of Recombinant Chinese Hamster Ovary Cells," *J. Biol. Chem.* 263(8): 3657-3663 (1988).

'216 Patent

Claims 54, 70 and 71 describe a methodology for the insertion of foreign DNA I into cells. Claim 73 describes a cell with characteristics produced using the methodology of claim 54. None of these claims describes the nature of DNA I that is inserted into the cell (i.e., which polypeptide the DNA I encodes). Claims 55-56 and 58-60 define the DNA I of claim 54 as encoding proteinaceous material, interferon protein, growth hormone, a clotting factor, and a viral antigen or antibody, respectively.

None of the claims in the '216 patent reports on whether the cell is competent to transcribe and translate DNA I. Even if one assumes that the host cell is competent to transcribe and translate DNA I, the claims do not report on whether the transcription and translation would be proper, nor whether the cell is competent to perform post-translational processing of a polypeptide encoded by DNA I.

'017 Patent

Claim 1 defines a Chinese Hamster Ovary (CHO) cell that comprises amplified foreign DNA I corresponding to a gene of interest, stably incorporated into the chromosomal DNA, and amplified DNA II. Claim 4 refers to claim 1 wherein DNA II encodes dihydrofolate reductase. None of these claims describes the nature of DNA I that is inserted into the cell. Claim 2 refers to the cell of claim 1 wherein foreign DNA I encodes a proteinaceous material, which is not associated with a selectable phenotype. Claim 3 refers to the cell of claim 2, wherein the proteinaceous material that the DNA encodes is interferon protein, insulin, a growth hormone, a clotting factor, a viral antigen, an antibody, or an enzyme.

Claims 1-4 do not report on whether the cell is competent to transcribe and translate DNA I. Even if one assumes that the cell is competent to transcribe and translate DNA I, the claims do not report on whether the transcription and translation would be proper, nor whether the cell is competent to perform post translational processing of a polypeptide encoded by DNA I.

Claim 5 describes the production of proteinaceous protein using the cell of claim 1. It is assumed that the terms *proteinaceous protein* and *proteinaceous material* are equivalent. The claim does not report on whether DNA I is accurately transcribed and translated. It also says nothing about the competence of the cell to perform post-translational modifications to the foreign polypeptide. Specifically, nothing is said as to whether the host cell is competent to glycosylate a foreign polypeptide or whether appropriate conditions exist for glycosylation to occur. It should be noted that no distinction is made as to whether the proteinaceous protein is a product of DNA II or DNA I. In addition, no indication is given as to whether the proteinaceous protein is secreted.

'665 Patent

Claim 1 describes a methodology for the insertion of foreign DNA into cells. Claims 12-13 describe host cells produced using the methodology of claim 1. The claims do not describe the nature of DNA I that is inserted into the host cell. Claims 2-11 describe variations on the methodology of claim 1. Only claims 5 and 6 describe the nature of DNA I inserted into the host cell.

None of the claims reports on whether the host cell is competent to transcribe and translate DNA I. Even if one assumes that the host cell is competent to transcribe and

translate DNA I, the claims do not report on whether the transcription and translation would be proper, nor whether the cell is competent to perform post-translational processing of a polypeptide encoded by DNA I.

Claim 14 describes the production of proteinaceous material via the use of the host cell of claim 1. It does not report whether DNA I is accurately transcribed and translated. It also says nothing about the competence of the cell to perform post-translational modifications to the foreign polypeptide. In particular, nothing is said about whether the host cell is competent to glycosylate a foreign polypeptide or whether appropriate conditions exist for glycosylation to occur. It should be noted that no distinction is made as to whether the proteinaceous material is a product of DNA II or DNA I. In addition, no indication is given as to whether the protein is secreted.

'275 Patent

Claim 19 describes the transformed CHO cell of any of claims 16-18, further comprising the "glycoprotein of interest." The host cell of claim 16 includes DNA I that encodes "a glycoprotein of interest." The host cell of claim 19 does not just contain DNA I; it also comprises "the glycoprotein of interest" itself. Because the "glycoprotein of interest" is present in the host cell, the cell of claim 19 must be competent to produce the "glycoprotein of interest" and must have been cultured under conditions suitable to allow the proper post-translational modifications to occur.

Conclusion:

Even if one assumes that all of the claims of the original Axel patents cited by the plaintiffs describe a host cell that can transcribe and translate the foreign gene into a

polypeptide, none of the claims cited by the plaintiffs addresses the issue of glycosylation. For the reasons discussed above, a person of skill in the art (either ordinary or extraordinary) in February 1980 would not have considered claim 19 of the '275 patent obvious in light of any claim of the original Axel patents. A person of skill in the art could not have had a reasonable expectation that he or she could construct a CHO host cell with the characteristics of the cell of claim 19 that was competent to produce the "glycoprotein of interest" of claim 19, and culture it under suitable conditions such that it would comprise the "glycoprotein of interest." To the contrary, when this was established, it was considered an unexpected result. The production of polypeptides in eukaryotic cells was an intriguing field to scientists, but there was nothing that provided the data, instructions or critical factors necessary for an individual of skill in the art to consider there to be a reasonable likelihood of success that the invention of claim 19 could be achieved.

There is an additional aspect of Claim 19 that is worth discussing. Claim 19 recites amplified DNA I and amplified DNA II. This means that there are multiple copies of DNA I and DNA II in the host cell. As described below, amplification is an aberrant or at least unusual event within a cell (indeed, it is often associated with cancer cells). Researchers in February 1980 would have been very concerned that the volume of foreign polypeptides encoded by all the amplified copies of the foreign DNA I and DNA II that could be produced by the host cells would overwhelm the processing apparatus of the cell and thus prevent the host cell from producing the "glycoprotein of interest." This concern can be illustrated with a hypothetical example. Assume that a host cell naturally produces a certain number of molecules in an hour. In February 1980, a person of ordinary skill would think that the processing apparatus of the host cell is therefore designed to produce and process a certain number of molecules, and in specific to perform a certain type and number

of post-translational modifications in an hour. This requires the use of various different enzymes and other materials, of which the cell has only a finite amount. If the cell has to contend suddenly with a greater number of molecules (as the result of the amplification of DNA I and DNA II and transcription/translation thereof), it would be unclear how the enzymes and supporting apparatus necessary for glycosylation would have been able to respond. A researcher in February 1980 would have been very concerned that the cell's processing apparatus would become gridlocked. The concept of multiple genes overloading the transcriptional, translational, and/or processing functions is sometimes referred to as "squelching." In fact, in 1985, researchers continued to view it as significant enough of an issue to merit discussion in a journal publication.[19] This is an additional reason why claim 19 is not obvious in light of any claim of the original Axel patents.

D. GENE AMPLIFICATION, STABILITY, AND LINKAGE

Claims 3, 5-14, and 16-19 of the '275 patent require that amplified DNA I and amplified DNA II are both stably incorporated into the chromosomal DNA of the host cell. In contrast, the claims of the '216, '665, and '017 patents recite at most the incorporation of amplified DNA I into the chromosomal DNA. Specifically, claims 1-5 of the '017 patent recite the stable incorporation of DNA I in the chromosomal DNA and claim 71 of the '216 patent recites the incorporation of DNA I in the chromosomal DNA. Thus, none of the claims of the original Axel patents recites the stable incorporation of DNA II into chromosomal DNA.

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[19] Kaufman, "Coamplification and Coexpression of Human Tissue-Type Plasminogen Activator and Murine Dihydrofolate Reductase Sequencs in Chinese Hamster Ovary Cells," Mol. & Cell. Biol., 5:7:1750-1759 (1985) (concluding that amplification did not impact processing).

The stable incorporation of DNA II would not have been obvious in light of any of the claims of the original Axel patents. In February 1980, researchers understood very little about gene amplification. Before February 1980, researchers had observed that certain cells were resistant to drugs that normally killed cells. For example, when one exposed a population of cells to methotrexate (mtx), a chemical that kills most cells, researchers observed that a small number of cells would survive. In the late 1970s, researchers determined that these cells survived because they had an amplified copy number of a particular gene that produces an enzyme that confers resistance to mtx. That enzyme is dihydrofolate reductase (DHFR).[20] These observations related generally to native genes already in the cells as contrasted with foreign genes inserted into the cells.[21]

By February 1980, gene amplification in cell culture had been observed phenomenologically, but the mechanics of amplification were poorly understood. The leading researcher in the field of gene amplification at that time was Professor Robert Schimke of Stanford University. In a series of research papers published just before the application date of the '275 patent, he reported that the way amplification works was

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[20] Schimke, R. *et al.*, "Gene Amplification and Drug Resistance in Cultured Murine Cells," *Science*, 202:1051-1055 (1978).

[21] In 1979, I published a paper with George Scangos and Lawrence Klobutcher that presented evidence of an increase in copy number of nucleic acids on a foreign chromosome inserted into a cell. Scangos, G., *et al.*, "Gene Amplification as a Concomitant to Chromosome Mediated Gene Transfer," in *Eucaryotic Gene Regulation* 445-456 (Axel *et al.* eds. 1979). The process of transferring foreign chromosomes to a cell is distinct from the strategy used in the Axel patents of transferring pieces of DNA (i.e., DNA-mediated gene transfer). The mechanism of action that led to the increased copy number was not then understood.

essentially unknown.[22] Even today, twenty-five years later, the mechanism of gene amplification is not completely understood.

In February 1980, there were conflicting observations about what caused amplification, the extent to which gene amplification persisted in the absence of "selection" (i.e., introduction of an agent, such as mtx, that would ordinarily kill the cell), and whether genes were amplified within chromosomes, outside chromosomes, or some combination of both. The vast majority of DNA in a normal cell is associated with the chromosomes. However, DNA need not necessarily be associated with chromosomes. Researchers studying amplification in the period before the Axel inventions recognized that the amplified gene copies were not always associated with chromosomal DNA of the cell. Instead, they could be located in extra-chromosomal elements termed double minutes (DMs).[23] DMs can

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[22] Schimke, R.T. *et al.*, "Amplification of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.* 42:655 (1978) ("The observation that under appropriate selection conditions the rate of synthesis of a specific protein can be altered by changes in the number of specific genes in cultured mammalian cells has, to the best of our knowledge, not been clearly documented previously. We know very little about the mechanisms of the process whereby this occurs . . . ."); Schimke, R.T. *et al.*, "Structure and localization of dihydrofolate reductase genes in methotrexate-resistant cultured cells" in *Eucaryotic Gene Regulation: ICN-UCLA Symposia on Molecular & Cellular Biology* 509 (Axel *et al.* eds. 1979) ("The mechanism for the amplification process is not understood at present."). *See also,* Alt, F. *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978); Schimke, R. *et al.*, "Gene Amplification and Drug Resistance in Cultured Murine Cells," *Science*, 202:1051-1055 (1978).

[23] Kaufman, R., *et al.*, "Amplified Dihydrofolate Reductase Genes in Unstably Methotrexate-Resistant Cells are Associated with Double Minute Chromosomes," *Proc. Natl. Acad. Sci. U.S.A.* 76(11):5669-5673 (1979); Schimke, R.T. *et al.*, "Studies on the Amplification of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.*, 43:1297-1303 (1979); Schimke, R.T. *et al.*, "Structure and localization of dihydrofolate reductase genes in methotrexate-resistant cultured cells" in *Eucaryotic Gene Regulation: ICN-UCLA Symposia on Molecular & Cellular Biology*, 499-510 (Axel *et al.* eds. 1979). *See also,* Alt, F. *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of

be functional. That is to say, a cell can transcribe and translate the genes located on DMs to produce a polypeptide, even though the DMs reside outside of the chromosome.

Given that the mechanism of amplification of endogenous genes was so poorly understood, there would have been nothing obvious or predictable about amplification in the context of the Axel patents. This is especially the case given the lack of data on the status of amplified foreign DNA in DNA mediated gene transfer systems. In February 1980, a scientist of ordinary skill in the art would not have been able to predict the status of the amplified transfected DNA. Researchers understood in February 1980 that DNA amplification, although a natural biological phenomenon, is essentially an unpredictable process. It is a process often seen in aberrant situations such as the imposition of drug resistance on a cell population or in unregulated atypical growth as seen in cancerous cell populations.[24] In February 1980, there was nothing more than a general understanding of amplification. This general understanding would make further experimentation appropriate, but would not have provided any data or instruction on critical factors sufficient for a person of skill in the art (whether ordinary or extraordinary skill) to consider there to be a reasonable likelihood of success of practicing claims 3, 5-14, and 16-19 of the '275 patent.

Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978); Schimke, R. *et al.*, "Gene Amplification and Drug Resistance in Cultured Murine Cells," *Science*, 202:1051-1055 (1978).

[24] Levan, G. *et al.*, "Double Minute Chromosomes are Not Centromeric Regions of the Host Chromosomes," *Hereditas* 83:1, 83-90 (1976); Levan, G. *et al.*, "Experimental Elimination and Recovery of Double Minute Chromosomes in Malignant Cell Populations," *Hereditas*, 86:1, 75-90 (1977); Balaban-Malenbaum, G. *et al.*, "Double Minute Chromosomes and the Homogeneously Staining Regions in Chromosomes of a Human Neuroblastoma Cell Line," *Science*, 198:739-741 (1977).

The Axel Patents

I have reviewed the declaration of Dr. Harvey Lodish and the charts submitted by the plaintiffs. They argue that various claims of the '216, '655, and '017 patents make obvious the stable incorporation of amplified DNA II, even though none of the claims of those patents recites the stable incorporation of DNA II. Their reasons include the following:

A: Regarding the '017 patent, plaintiffs argue that "Foreign DNA I is stably incorporated into chromosomal DNA and both DNA I and DNA II are amplified. Thus, a cell that contains amplified DNA I that is stably incorporated would include DNA I and DNA II stably incorporated into the chromosome." Dr. Lodish, in his declaration, makes essentially the same assertion.

B: Regarding the '216 patent, plaintiffs argue that "Culturing cells in 'the presence of successively elevated concentrations of an agent permitting survival or identification of eucaryotic cells' enables one to select cells with amplified DNA II. Because DNA I is linked to DNA II, any cell that contains amplified DNA II will contain amplified DNA I. A person of ordinary skill in the art at the time of the invention would have expected that a cell that contains amplified DNA I linked to amplified DNA II would include DNA I and DNA II stably incorporated into chromosomal DNA."[25] Plaintiffs additionally reference the fact that claim 71 of the '216 patent recites the incorporation of DNA I in the chromosomal DNA.

Plaintiffs' statements regarding the '017 patent are scientifically inaccurate. It is not the case that DNA II is necessarily incorporated into the chromosome merely because

[25] Reiterating this argument, plaintiffs also make the following statement: "'[B]oth DNA I and DNA II being stably incorporated' is not patentably distinct in view of claim

DNA II and DNA I are amplified and DNA I is stably incorporated into the chromosome. In fact, scientific observations both in February 1980 and subsequently establish that amplified DNA II can reside within extra-chromosomal elements such as DMs.[26]

Plaintiffs' statements regarding the '216 patent are also incorrect, because they ignore the fact that there was a fundamental lack of understanding in 1980 about how amplification occurs both for genes native to a cell and genes inserted into a cell. In February 1980, researchers in the field were proposing a number of different explanations for the mechanism of amplification, none of which could be definitively excluded or confirmed. Plaintiffs assume that because claim 54 of the '216 patent discloses that DNA I and DNA II are linked at one point, it would have been obvious in February 1980 that they would remain linked together after amplification and that both would stably incorporate into the chromosomal DNA. However, in February 1980, the data on amplification suggested that it was to a large extent an unpredictable process. For example, some cells appeared to have a large number of amplified copies of DNA, while other cells of the same type failed to amplify DNA at all. Further complicating matters, the mechanism for the loss and

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54/55s recitation of culturing under conditions which yield cells acquiring multiple copies of DNA I and DNA II."

[26] Carroll, S. M. *et al.*, "Characterization of an Episome Produced in Hamster Cells That Amplify a Transfected CAD Gene at High Frequency: Functional Evidence for a Mammalian Replication Origin," *Mol. Cell. Biol.* 7(5):1740-1750 (1987); Roberts, J.M. *et al.*, "A Structure for Amplified DNA," *Cell*, 33:53-63 (1983); Schimke, R.T. *et al.*, "Studies on the Amplification of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.*, 43:1297-1303 (1979); Schimke, R.T. *et al.*, "Structure and localization of dihydrofolate reductase genes in methotrexate-resistant cultured cells" in *Eucaryotic Gene Regulation: ICN-UCLA Symposia on Molecular & Cellular Biology*, 499-510 (Axel *et al.* eds. 1979); Kaufman, R., *et al.*, "Amplified Dihydrofolate Reductase Genes in Unstably Methotrexate-Resistant Cells are Associated with Double Minute Chromosomes," *Proc. Natl. Acad. Sci. U.S.A.* 76(11):5669-5673 (1979); Alt, F. *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in

maintenance of amplified DNA in certain was not completely understood.[27] There was not sufficient experimental evidence or guidance in the field to allow scientists at any level of skill to have confidence about what would happen when and if two pieces of linked foreign DNA were amplified, even if one knew (as in claim 71 of the '216 patent) that DNA I is incorporated into the chromosomal DNA.

The question of what would happen after amplification when DNA I and DNA II are linked was not just unclear because of a lack of experimental data in February 1980. To the contrary, depending on the theory of amplification one considered, it would have been highly likely that DNA I and DNA II would not be linked together after amplification. In February 1980, Schimke postulated that gene amplification might have occurred as a consequence of reverse transcription of mRNA into DNA.[28] In reverse transcription, it would have been theorized that mRNA is turned back into a form of DNA. If this was in fact the mechanism of amplification—and in February of 1980 there would be no way of discounting it—then one would assume that two pieces of DNA linked together either before

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Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978).

[27] *See generally,* Schimke, R.T. *et al.,* "Studies on the Amplification of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.*, 43:1297-1303 (1979); Kaufman, R., *et al.*, "Amplified Dihydrofolate Reductase Genes in Unstably Methotrexate-Resistant Cells are Associated with Double Minute Chromosomes," *Proc. Natl. Acad. Sci. U.S.A.* 76(11):5669-5673 (1979); Alt, F. *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978); Schimke, R.T. *et al.,* "Amplification of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.* 42:649-657 (1978); Schimke, R.T. *et al.,* "Structure and localization of dihydrofolate reductase genes in methotrexate-resistant cultured cells" in *Eucaryotic Gene Regulation: ICN-UCLA Symposia on Molecular & Cellular Biology*, 499-510 (Axel *et al.* eds. 1979).

[28] Alt, F. *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978).

insertion into a cell or just after insertion into a cell would not stay linked together following amplification. This is because the transcription of the linked pieces of DNA would produce unlinked mRNA transcripts, which the cell would then read to produce unlinked copies of two pieces of DNA.

Conclusion

Given the dearth of experimental data on amplification of foreign genes in a cell, the fundamental lack of understanding of the mechanism of action of amplification, and the aberrant nature of amplification, there is nothing disclosed in any claim of the original Axel patents, or in scientific literature as of February 1980, that would have made obvious to a person of any level of skill in the art that DNA II would be stably incorporated into chromosomal DNA merely because DNA I and DNA II are amplified. This is the case even if DNA I and DNA II are linked together at one time, and even if DNA I is stably incorporated into the chromosomal DNA. Therefore, claims 3, 5-14, and 16-19 are not obvious in light of any claim of the original Axel patents.

Finally, it is important to emphasize that the presence of DNA II in the chromosomal DNA of a host cell is not by any means a minor or unimportant difference between the claims of the original Axel patents and the claims of the '275 patent. To the contrary, it is a valuable difference. When the foreign DNA is outside of the chromosome, it means that the host cell (and its progeny) may be genetically unstable. From a commercial standpoint, this is undesirable. Host cells that are used to produce a given drug should remain constant in their genetic characteristics. If the host cell changes, it could change the character of the products it expresses in an unpredictable manner. The '275 patent claims host cells that are genetically stable.

E. ADDITIONAL DIFFERENCES

There are some additional differences between the claims of the '275 patent and the claims of the '216 and '665 patents cited by plaintiffs that are relevant. The claims of the '216 and '665 patents do not disclose the use of a CHO cell. They disclose the use of either a mammalian or a eukaryotic cell. A person of ordinary skill in the art as of February 1980 would not have considered it obvious that a CHO cell could be used successfully to practice the inventions claimed in the '275 patent. The main references relating to mammalian cell DNA-mediated transformation – those from the Axel lab, and those from the O'Malley, Weissmann and Berg labs – did not use CHO cells. They generally used special types of mouse and monkey cells. In addition, papers from those labs indicated the same thing: it was difficult to insert foreign DNA into eukaryotic host cells successfully.[29] A person of ordinary skill in the art would thus have had serious doubts about the general applicability of the transformation systems disclosed in the '216 and '665 patents. If a person of ordinary skill wanted to attempt to practice claim 19, and have a reasonable probability of success, he or she would not have wanted to use a cell type that had been proven consistently effective in the system – such as a CHO cell.[30] Instead, that person would have used the same types

[29] See, e.g., Wigler, M. *et al.*, "Biochemical Transfer of Single-Copy Eucaryotic Genes Using Total Celluar DNA as Donor," *Cell* 14:725-731 (1978); Mantei, N. *et al.*, "Rabbit β-globin mRNA production in mouse L cells transformed with cloned rabbit beta-globin chromosomal DNA," *Nature*, 281:40-46 (1979); Lai, E., *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene,"*Proc. Nat. Acad. Sci. U.S.A.* 77(1):244-248 (1980); Mulligan, R.C, *et al.*, "Synthesis of rabbit β-globin in cultured monkey kidney cells following infection with a SV40 P-globin recombinant genome," *Nature* 277:108-114 (1979).

[30] In 1980, Srinivasan and Lewis published an article describing their attempts to transfer genetic material into a CHO cell line. It is unclear to me whether this article was available to researchers by February 1980. Srinivasan, P.R. *et al.*, "Transfer of the Dihydrofolate Reductase Gene into Mammalian Cells Using Metaphase Chromosomes or Purified DNA," in *Introduction of Macromolecules into Viable Mammalian Cells* 27-45

of cells generally used by the Axel lab – special types of mouse cells. This would have deterred a person of ordinary skill from choosing a CHO cell. This is, in part, because the references do not conclusively define what, if any, characteristics of the mouse cells the Axel, O'Malley, Weissmann labs used made them successful candidates. Thus, a person of skill in the art would not have considered there to be a reasonable likelihood of success that the claims of the '275 could be practiced using CHO cells in light of any claim of the '216 or '665 patent.

In addition, it is noteworthy that none of the claims of the '665 patent discloses the amplification or stable incorporation of DNA I or DNA II. The ability to achieve amplification and stable incorporation of foreign DNA I and foreign DNA II in a DNA-mediated transformation system was a novel insight in February 1980. As discussed above, there was only a very limited and general understanding of the phenomenon of amplification. Based on this general understanding, no person of either ordinary or extraordinary skill in the art as of February 1980 would have considered there to be a reasonable likelihood of success that both DNA I and DNA II could be amplified and stably incorporated into the chromosomal DNA in light of any claim of the '665 patent.

G. GENERAL SUBJECTS OF TESTIMONY

In addition to the opinions discussed above, I will be prepared at trial to discuss general scientific principles and concepts associated with the subject matter of the Axel

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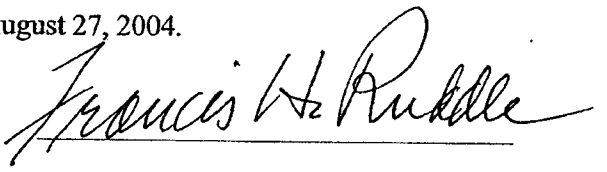
(Baserga, R. *et al.*, eds. 1980). However, if it was, it would actually provide further evidence that the use of CHO cells to practice the inventions claimed in the '275 patent would not have been obvious. The article indicates that Srinivasan and Lewis were having difficulty transferring genetic material into CHO cells. Knowing this information, an individual of ordinary skill would have been further deterred from using CHO cells to attempt to obtain the host cells claimed in the '275 patent.

patents. I also plan on discussing the methodology the Axel patents teach to obtain cells with certain foreign DNA. In addition, once the plaintiffs have presented their position on the '275 patent, I plan on responding to their position if necessary. Finally, I am continuing to analyze issues discussed herein. I reserve the right to expand on observations in my report and to present additional information and analysis to the extent appropriate or necessary. I may also present illustrations of the biological processes discussed in the report, the content of which will reproduce the descriptions of those processes given herein.

III. OTHER EXPERT TESTIMONY, COMPENSATION FOR TIME, MATERIALS RELIED ON

I have never testified at trial or deposition as an expert witness. I am being compensated for my time at the rate of $500 an hour. Attached as Exhibit B is a list of the materials I have and/or may rely on in the future as part of my expert testimony.

Executed at Los Angeles, California on August 27, 2004.



Francis H. Ruddle

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

IN RE COLUMBIA UNIVERSITY PATENT LITIGATION	MDL No. 1592 (MLW) This Document Relates To All Actions

EXPERT REPORT OF PROFESSOR FRANCIS H. RUDDLE SUBMITTED ON BEHALF OF THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

the roles they play in an organism.[9] Different cell types in an organism frequently express different types of polypeptides, and because of this, the types of post-translational modifications they normally perform are different. A person of ordinary skill in the art in February 1980 would have been extremely concerned about whether a host cell that is not known to ordinarily perform a particular post-translational modification on a particular polypeptide (i.e., the foreign polypeptide) would have the capability to perform the necessary post-translational modifications to that foreign polypeptide. In fact, there would have been substantial concern that a host cell might not recognize what to do with a foreign polypeptide, unless there were experimental data that established otherwise.[10]

Moreover, there was experimental evidence by February 1980 suggesting that different cells produce different glycosylation patterns on the same polypeptide.[11] In fact,

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"Observations of de novo clones of cytogentically aberrant cells in primary fibroblast cell strains from phenotypically normal women," *Am. J. Hum. Genet.* 27(2):190-197 (1975).

[9] *See, e.g.*, Muramatsu, T. *et al.*, "Carbohydrate structures and cell differentiation: Unique properties of fucosyl-glycopeptides isolated from embryonal carcinoma cells," *Proc. Natl. Acad. Sci. U.S.A.* 75(5):2315-2319 (1978); Muramatsu, T. *et al.*, "Characterization of glycopeptides isolated from membranes of F9 embryonal carcinoma cells," *Biochim. Biophys. Acta*, 587:392-406 (1979).

[10] A related concern would be whether a researcher, in the laboratory, could supply the specific nutrients and materials a cell might require to perform a particular post-translational modification. When part of an organism, the cell might obtain the specific components from other cells in the organism or its natural environment. In a laboratory, it might not have this ability.

[11] *See, generally,* Kornfeld, R. *et al.*, "Comparative Aspects of Glycoprotein Structure," in *Annual Review of Biochemistry* Vol. 45, 217-237 (Snell *et al* eds. 1976); Moczar, E., "Differentiation of the polysaccharide side-chains of glycoproteins by a fingerprinting technique: heterogeneity of human gastric mucin," *J. Chromatogr. Biomed. Appl.*, 181:108-114 (1980); Marshall, R., "Some Observations on Why Many Proteins are Glycosylated," *Biochem. Soc. Trans.* 7(4):800-805 (1979). *See also* Clamp, J.R. *et al.*, "Heterogeneity of glycopeptides from a homogeneous immunoglobulin," *Biochem. J.*, 100:35c-36c (1966); Gottschalk, A., "Biosynthesis of glycoproteins and its relationship to heterogeneity," *Nature* 222:452-454 (1969); Spiro, R.G., "Glycoproteins," *Adv. Protein Chem.* 27:349-467 (1973).

of post-translational modifications in an hour. This requires the use of various different enzymes and other materials, of which the cell has only a finite amount. If the cell has to contend suddenly with a greater number of molecules (as the result of the amplification of DNA I and DNA II and transcription/translation thereof), it would be unclear how the enzymes and supporting apparatus necessary for glycosylation would have been able to respond. A researcher in February 1980 would have been very concerned that the cell's processing apparatus would become gridlocked. The concept of multiple genes overloading the transcriptional, translational, and/or processing functions is sometimes referred to as "squelching." In fact, in 1985, researchers continued to view it as significant enough of an issue to merit discussion in a journal publication.[19] This is an additional reason why claim 19 is not obvious in light of any claim of the original Axel patents.

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maintenance of amplified DNA in certain cells was not completely understood.[27] There was not sufficient experimental evidence or guidance in the field to allow scientists at any level of skill to have confidence about what would happen when and if two pieces of linked foreign DNA were amplified, even if one knew (as in claim 71 of the '216 patent) that DNA I is incorporated into the chromosomal DNA.

The question of what would happen after amplification when DNA I and DNA II are linked was not just unclear because of a lack of experimental data in February 1980. To the contrary, depending on the theory of amplification one considered, it would have been highly likely that DNA I and DNA II would not be linked together after amplification. In February 1980, Schimke postulated that gene amplification might have occurred as a consequence of reverse transcription of mRNA into DNA.[28] In reverse transcription, it would have been theorized that mRNA is turned back into a form of DNA. If this was in fact the mechanism of amplification—and in February of 1980 there would be no way of discounting it—then one would assume that two pieces of DNA linked together either before

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Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978).

[27] *See generally,* Schimke, R.T. *et al.,* "Studies on the Amplification of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.*, 43:1297-1303 (1979); Kaufman, R., *et al.*, "Amplified Dihydrofolate Reductase Genes in Unstably Methotrexate-Resistant Cells are Associated with Double Minute Chromosomes," *Proc. Natl. Acad. Sci. U.S.A.* 76(11):5669-5673 (1979); Alt, F. *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978); Schimke, R.T. *et al.,* "Amplification of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.* 42:649-657 (1978); Schimke, R.T. *et al.,* "Structure and localization of dihydrofolate reductase genes in methotrexate-resistant cultured cells" in *Eucaryotic Gene Regulation: ICN-UCLA Symposia on Molecular & Cellular Biology*, 499-510 (Axel *et al.* eds. 1979).

[28] Alt, F. *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978).

E. ADDITIONAL DIFFERENCES

There are some additional differences between the claims of the '275 patent and the claims of the '216 and '665 patents cited by plaintiffs that are relevant. The claims of the '216 and '665 patents do not disclose the use of a CHO cell. They disclose the use of either a mammalian or a eukaryotic cell. A person of ordinary skill in the art as of February 1980 would not have considered it obvious that a CHO cell could be used successfully to practice the inventions claimed in the '275 patent. The main references relating to mammalian cell DNA-mediated transformation – those from the Axel lab, and those from the O'Malley, Weissmann and Berg labs – did not use CHO cells. They generally used special types of mouse and monkey cells. In addition, papers from those labs indicated the same thing: it was difficult to insert foreign DNA into eukaryotic host cells successfully.[29] A person of ordinary skill in the art would thus have had serious doubts about the general applicability of the transformation systems disclosed in the '216 and '665 patents. If a person of ordinary skill wanted to attempt to practice claim 19, and have a reasonable probability of success, he or she would not have wanted to use a cell type that had not been proven consistently effective in the system – such as a CHO cell.[30] Instead, that person would have used the

[29] See, e.g., Wigler, M. *et al.*, "Biochemical Transfer of Single-Copy Eucaryotic Genes Using Total Cellular DNA as Donor," *Cell* 14:725-731 (1978); Mantei, N. *et al.*, "Rabbit ß-globin mRNA production in mouse L cells transformed with cloned rabbit beta-globin chromosomal DNA," *Nature*, 281:40-46 (1979); Lai, E., *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene,"*Proc. Nat. Acad. Sci. U.S.A.* 77(1):244-248 (1980); Mulligan, R.C, *et al.*, "Synthesis of rabbit ß-globin in cultured monkey kidney cells following infection with a SV40 P-globin recombinant genome," *Nature* 277:108-114 (1979).

[30] In 1980, Srinivasan and Lewis published an article describing their attempts to transfer genetic material into a CHO cell line. It is unclear to me whether this article was available to researchers by February 1980. Srinivasan, P.R. *et al.*, "Transfer of the Dihydrofolate Reductase Gene into Mammalian Cells Using Metaphase Chromosomes or Purified DNA," in *Introduction of Macromolecules into Viable Mammalian Cells* 27-45

same types of cells generally used by the Axel lab – special types of mouse cells. This would have deterred a person of ordinary skill from choosing a CHO cell. This is, in part, because the references do not conclusively define what, if any, characteristics of the mouse cells the Axel, O'Malley, Weissmann labs used made them successful candidates. Thus, a person of skill in the art would not have considered there to be a reasonable likelihood of success that the claims of the '275 could be practiced using CHO cells in light of any claim of the '216 or '665 patent.

In addition, it is noteworthy that none of the claims of the '665 patent discloses the amplification or stable incorporation of DNA I or DNA II. The ability to achieve amplification and stable incorporation of foreign DNA I and foreign DNA II in a DNA-mediated transformation system was a novel insight in February 1980. As discussed above, there was only a very limited and general understanding of the phenomenon of amplification. Based on this general understanding, no person of either ordinary or extraordinary skill in the art as of February 1980 would have considered there to be a reasonable likelihood of success that both DNA I and DNA II could be amplified and stably incorporated into the chromosomal DNA in light of any claim of the '665 patent.

F. GENERAL SUBJECTS OF TESTIMONY

In addition to the opinions discussed above, I will be prepared at trial to discuss general scientific principles and concepts associated with the subject matter of the Axel

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(Baserga, R. *et al.*, eds. 1980). However, if it was, it would actually provide further evidence that the use of CHO cells to practice the inventions claimed in the '275 patent would not have been obvious. The article indicates that Srinivasan and Lewis were having difficulty transferring genetic material into CHO cells. Knowing this information, an individual of ordinary skill would have been further deterred from using CHO cells to attempt to obtain the host cells claimed in the '275 patent.

UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MASSACHUSETTS

IN RE COLUMBIA UNIVERSITY PATENT LITIGATION	MDL No. 1592 (MLW) This Document Relates To All Actions

EXPERT REPORT OF PROFESSOR FRANCIS H. RUDDLE SUBMITTED ON BEHALF OF THE ~~TRUSTEESS~~TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK

types of polypeptides, and because of this, the types of post-translational modifications they normally perform are different. A person of ordinary skill in the art in February 1980 would have been extremely concerned about whether a host cell that is not known to ordinarily perform a particular post-translational modification on a particular polypeptide (i.e., the foreign polypeptide) would have the capability to perform the necessary post-translational modifications to that foreign polypeptide. In fact, there would have been substantial concern that a host cell might not recognize what to do with a foreign polypeptide, unless there were experimental data that established otherwise.[10]

Moreover, there was experimental evidence by February 1980 suggesting that different cells produce different glycosylation patterns on the same polypeptide.[11] In fact, there was evidence that an organism would sometimes produce both non-glycosylated and glycosylated forms of the same molecule.[12] Researchers at the time would have accepted that different cells may treat the same polypeptide differently for post-translational modification

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"~~Charaterization~~Characterization of glycopeptides isolated from membranes of F9 ~~emryonal~~embryonal carcinoma cells," *Biochim. Biophys. Acta*, 587:392-406 (1979).

[10] A related concern would be whether a researcher, in the laboratory, could supply the specific nutrients and materials a cell might require to perform a particular post-translational modification. When part of an organism, the cell might obtain the specific components from other cells in the organism or its natural environment. In a laboratory, it might not have this ability.

[11] *See, generally,* Kornfeld, R. *et al.*, "Comparative Aspects of Glycoprotein Structure," in *Annual Review of Biochemistry* Vol. 45, 217-237 (Snell *et al* eds. 1976); Moczar, E., "Differentiation of the polysaccharide side-chains of glycoproteins by a fingerprinting technique: heterogeneity of human gastric mucin," *J. Chromatogr. Biomed. Appl.*, 181:108-114 (1980); Marshall, R., "Some Observations on Why Many Proteins are Glycosylated," *Biochem. Soc. Trans.* 7(4):800-805 (1979). *See also* Clamp, J.R. *et al.*, "Heterogeneity of glycopeptides from a homogeneous immunoglobulin," *Biochem. J.*, 100:35c-36c (1966); Gottschalk, A., "Biosynthesis of glycoproteins and its relationship to heterogeneity," *Nature* 222:452-454 (1969); Spiro, R.G., "Glycoproteins," *Adv. Protein Chem.* 27:349-467 (1973).

DNA II and transcription/translation thereof), it would be unclear how the enzymes and supporting apparatus necessary for glycosylation would have been able to respond. A researcher in February 1980 would have been very concerned that the cell's processing apparatus would become gridlocked. The concept of multiple genes overloading the transcriptional, translational, and/or processing functions is sometimes referred to as "squelching." In fact, in 1985, researchers continued to view it as significant enough of an issue to merit discussion in a journal publication.[19] This is an additional reason why claim 19 is not obvious in light of any claim of the original Axel patents.

D. GENE AMPLIFICATION, STABILITY, AND LINKAGE

Claims 3, 5-14, and 16-19 of the '275 patent require that amplified DNA I and amplified DNA II are both stably incorporated into the chromosomal DNA of the host cell. In contrast, the claims of the '216, '665, and '017 patents recite at most the incorporation of amplified DNA I into the chromosomal DNA. Specifically, claims 1-5 of the '017 patent recite the stable incorporation of DNA I in the chromosomal DNA and claim 71 of the '216 patent recites the incorporation of DNA I in the chromosomal DNA. Thus, none of the claims of the original Axel patents recites the stable incorporation of DNA II into chromosomal DNA.

The stable incorporation of DNA II would not have been obvious in light of any of the claims of the original Axel patents. In February 1980, researchers understood very little about gene amplification. Before February 1980, researchers had observed that certain cells were

[19] Kaufman, "Coamplification and Coexpression of Human Tissue-Type Plasminogen Activator and Murine Dihydrofolate Reductase ~~Sequenes~~**Sequences** in Chinese Hamster Ovary Cells," *Mol. & Cell. Biol.*, 5:(7): 1750-1759 (1985) (concluding that amplification did not impact processing).

Plaintiffs' statements regarding the '216 patent are also incorrect, because they ignore the fact that there was a fundamental lack of understanding in 1980 about how amplification occurs both for genes native to a cell and genes inserted into a cell. In February 1980, researchers in the field were proposing a number of different explanations for the mechanism of amplification, none of which could be definitively excluded or confirmed. Plaintiffs assume that because claim 54 of the '216 patent discloses that DNA I and DNA II are linked at one point, it would have been obvious in February 1980 that they would remain linked together after amplification and that both would stably incorporate into the chromosomal DNA. However, in February 1980, the data on amplification suggested that it was to a large extent an unpredictable process. For example, some cells appeared to have a large number of amplified copies of DNA, while other cells of the same type failed to amplify DNA at all. Further complicating matters, the mechanism for the loss and maintenance of amplified DNA in certain **cells** was not completely understood.[27] There was not sufficient experimental evidence or guidance in the field to allow scientists at any level of skill to have confidence

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cultured cells" in *Eucaryotic Gene Regulation: ICN-UCLA Symposia on Molecular & Cellular Biology*, 499-510 (Axel *et al.* eds. 1979); Kaufman, R., *et al.*, "Amplified Dihydrofolate Reductase Genes in Unstably Methotrexate-Resistant Cells are Associated with Double Minute Chromosomes," *Proc. Natl. Acad. Sci. U.S.A.* 76(11):5669-5673 (1979); Alt, F. *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978).

[27] *See generally,* Schimke, R.T. *et al.*, "Studies on the Amplification of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.*, 43:1297-1303 (1979); Kaufman, R., *et al.*, "Amplified Dihydrofolate Reductase Genes in Unstably Methotrexate-Resistant Cells are Associated with Double Minute Chromosomes," *Proc. Natl. Acad. Sci. U.S.A.* 76(11):5669-5673 (1979); Alt, F. *et al.*, "Selective Multiplication of Dihydrofolate Reductase Genes in Methotrexate-resistant Variants of Cultured Murine Cells," *J. Biol. Chem.* 253(5):1357-1370 (1978); Schimke, R.T. *et al.*, "Amplification of Dihydrofolate Reductase Genes in Methotrexate-resistant Cultured Mouse Cells," *Cold Spring Harb. Symp. Quant. Biol.* 42:649-657 (1978); Schimke, R.T. *et al.*, "Structure and localization of dihydrofolate reductase genes in methotrexate-resistant cultured

Weissmann and Berg labs – did not use CHO cells. They generally used special types of mouse and monkey cells. In addition, papers from those labs indicated the same thing: it was difficult to insert foreign DNA into eukaryotic host cells successfully.[29] A person of ordinary skill in the art would thus have had serious doubts about the general applicability of the transformation systems disclosed in the '216 and '665 patents. If a person of ordinary skill wanted to attempt to practice claim 19, and have a reasonable probability of success, he or she would not have wanted to use a cell type that had **not** been proven consistently effective in the system – such as a CHO cell.[30] Instead, that person would have used the same types of cells generally used by the Axel lab – special types of mouse cells. This would have deterred a person of ordinary skill from choosing a CHO cell. This is, in part, because the references do not conclusively define what, if any, characteristics of the mouse cells the Axel, O'Malley, Weissmann labs used made them successful candidates. Thus, a person of skill in the art would not have considered there to be a reasonable likelihood of success that the claims of the '275 could be practiced using CHO cells in light of any claim of the '216 or '665 patent.

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[29] See, e.g., Wigler, M. *et al.*, "Biochemical Transfer of Single-Copy Eucaryotic Genes Using Total ~~Cellular~~**Cellular** DNA as Donor," *Cell* 14:725-731 (1978); Mantei, N. *et al.*, "Rabbit ß-globin mRNA production in mouse L cells transformed with cloned rabbit beta-globin chromosomal DNA," *Nature*, 281:40-46 (1979); Lai, E., *et al.*, "Ovalbumin is Synthesized in Mouse Cells Transformed with the Natural Chicken Ovalbumin Gene,"*Proc. Nat. Acad. Sci. U.S.A.* 77(1):244-248 (1980); Mulligan, R.C, *et al.*, "Synthesis of rabbit ß-globin in cultured monkey kidney cells following infection with a SV40 P-globin recombinant genome," *Nature* 277:108-114 (1979).

[30] In 1980, Srinivasan and Lewis published an article describing their attempts to transfer genetic material into a CHO cell line. It is unclear to me whether this article was available to researchers by February 1980. Srinivasan, P.R. *et al.*, "Transfer of the Dihydrofolate Reductase Gene into Mammalian Cells Using Metaphase Chromosomes or Purified DNA," in *Introduction of Macromolecules into Viable Mammalian Cells* 27-45 (Baserga, R. *et al.*, eds. 1980). However, if it was, it would actually provide further evidence that the use of CHO cells to practice the inventions claimed in the '275 patent would not have been obvious. The article indicates that Srinivasan and Lewis were having difficulty transferring genetic material into CHO cells. Knowing this information, an individual of

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~~G~~F. GENERAL SUBJECTS OF TESTIMONY

In addition to the opinions discussed above, I will be prepared at trial to discuss general scientific principles and concepts associated with the subject matter of the Axel patents. I also plan on discussing the methodology the Axel patents teach to obtain cells with certain foreign DNA. In addition, once the plaintiffs have presented their position on the '275 patent, I plan on responding to their position if necessary. Finally, I am continuing to analyze issues discussed herein. I reserve the right to expand on observations in my report and to present additional information and analysis to the extent appropriate or necessary. I may also present illustrations of the biological processes discussed in the report, the content of which will reproduce the descriptions of those processes given herein.

III. OTHER EXPERT TESTIMONY, COMPENSATION FOR TIME, MATERIALS RELIED ON

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ordinary skill would have been further deterred from using CHO cells to attempt to obtain the